REMARKS

Upon entry of the present amendment, Claims 43-66 will remain pending (Claims 1-42 having been previously cancelled).

Claims 48, 51, 52, 55-60 and 65 are withdrawn from consideration.

Claims 43-47, 49, 50, 53, 54, 61-64 and 66 are rejected. Applicants have amended the pending rejected and several of the withdrawn from examination claims by omitting SEQ ID NO: 12, 14 and SEQ ID NO: 18 and from the pertinent claims. SEQ ID NO:12 and 18 are deleted because these sequences do nor correspond to a subsequence of SEQ ID NO:11. SEQ ID NO:14 is deleted because it is substantially identical to SEQ ID NO:13 (same aside from a GT dinucleotide in the 3' end (see paragraph [0052] of specification)). Also, SEQ ID NO:8 and 9 are deleted from claim 50. Otherwise the claims currently under examination have not been changed. An indication of allowance of all claims based on the remarks herein and the Declaration by two of the inventors is respectfully requested.

This Reply is fully responsive to the Office Action mailed on July 15, 2008. All of the pending claims with the exception of those withdrawn from consideration stand rejected under 35 USC 112 first paragraph. This rejection should be vacated in its entirety for the reason addressed infra.

Claim Rejections - 35 USC § 112

Claims 43-47, 49, 50, 53, 54, 61, 63, 64 and 66 stand rejected under 35 USC§112 first paragraph. The basis of the maintained rejection is the Examiner's position that the specification only enables a method of diagnosing a poor prognosis of B-CLL by detecting the presence of the exon 2/exon 3 splice junction in an AMB-1 transcript, but allegedly does not enable a method for detecting a subtype of B-CLL with poor prognosis by detecting any expression product within SEQ ID NO:12-18 (now SEQ ID NOs: 13-17).

In particular the Office Action states as follows:

"The [prior Applicant's] response states that data shown in figure 3 of the specification clearly establishes that Applicants are in possession of and sufficiently describe a method of reproducibly detecting AMB-1 transcripts in cell extracts by means of Northern blotting, described in [0247]. It should be noted that in this Figure the left hand lanes UPN1, UPN4 and UPN7 are samples from B-CLL patients with un-mutated IgVH genes, whereas the lanes UPN19, UPN9, UPN10, UPN13, and UPN21 are samples from B-CLL patients with mutated IgVH genes. On the right are samples from tissues of healthy persons. The blot is probed with a fragment of Exon 3 (SEQ ID NO:16). The data clearly confirms the presence of at least three transcripts in samples of subtype of B-cells with un-mutated IgVH genes-one transcript with a size less than 1.8 kb (18S RNA marker), another larger transcript between the 1.8 kb 18S RNA marker and another transcript below the 5.0 kb 28S RNA marker. The third long transcript in the top of the blot corresponds to the primary un-spliced. This Northern blot confirms the transcripts comprising Exon 3 correlate to and may be detected in B-CLL patients with un-mutated IgVH genes. On the other hand, transcripts comprising Exon 3 is not detected in samples from B-CLL patients with mutated IgVH genes and samples from healthy persons. Thus, the presence of AMB-1 transcripts comprising Exon 3 (SEQ ID NO:16) correlates to the presence of B-cells with un-mutated IgVH genes (see response pages 10-12).

In response to this argument, the Northern blot in Figure 3 was probed with a region of Exon 3, thus detecting the Exon 3 region of RNA expressed in various samples. in this instance, the transcript was detected in mutated B-Cll patients but not in unmutated B-CLL patients. This, detect of Exon 3 identifies a subpopulation of B-cell patients, but there is no evidence that the entire transcript of SEQ ID NO:11 is absent from B-cell patients or a subtype of B-cell patients. Since the blot does not include any control samples or markers it is unclear exactly what the different transcripts are that are identified by the Exon 3 probe.

Additionally, as set forth in the previous Office action, the sequences of SEQ ID NO: 11 do not produce a consensus sequence, therefore detection of SEQ ID NO:11 would not necessarily detect a region of SEQ ID NOs: 12-18."

The position of the Examiner is respectfully traversed based on the remarks below and the supporting data and the information contained in the declaration provided herewith. However, Applicants respectfully submit that even in the absence of this additional data there is sufficient information and data in the specification, when this information and data is properly analyzed, which would enable one skilled in the art to practice the claimed methods absent undue experimentation for detecting a particular virulent genetic subtype of B cell chronic lymphocytic leukaemia (B-CLL) based on the detection of the presence or absence of the expression of specific sequences as claimed herein.

As described in the as-filed specification and consistent with the claims, the present invention generally relates to a diagnostic method for the identification of a specific subtype of B-CLL patients (un-mutated) using AMB-1 as marker.

The presence of AMB-1 correlates with the un-mutated subtype. As earlier explained the data contained in the Northern blot of Figure 3 and the RNA dot blot of Figure 8 substantiate the efficacy of the claimed methods and indeed provide proof of principle, namely that the claimed methods can reliably be used as a mean of detecting whether an individual has or lacks a specific subtype of B-CLL. The fact that a primary transcript, identified by a probe comprising a fragment of Exon-3, is present in the unmutated, but absent in the mutated would substantiate that any spliced transcripts will also be absent simply due to the fact that they originate from the parent primary transcript.

Again Applicants respectfully submit that the data shown in figure 3 of the present application clearly establishes that Applicants are in possession of and sufficiently describe a method of reproducibly detecting AMB-1 transcripts in cell extracts by means of Northern blotting, described in [0247]. It should be noted that in this Figure the left hand lanes UPN1, UPN4, and UPN7 are samples from B-CLL patients with un-mutated IgV_H genes, whereas the lanes UPN19, UNP9, UPN10, UPN13, UPN21 are samples from B-CLL patients with mutated IgV_H genes. On the right are samples from tissues of healthy persons. The blot is probed with a fragment of Exon 3 (SEQ ID No: 16). The data clearly confirms the presence of at least three transcripts in samples of subtype of B-cells with un-mutated IgV_H genes – one transcript with a size less than the 1.8 kb (18S RNA marker), another larger transcript between the 1.8 kb 18S RNA marker and another transcript

below the 5.0 kb 28S RNA marker. The third long transcript in the top of the blot corresponds to the primary un-spliced.

This Northern blot confirms that transcripts comprising Exon 3 correlate to and may be detected in B-CLL patients with un-mutated IgVH genes. On the other hand, transcripts comprising Exon 3 is not detected in samples from B-CLL patients with mutated IgV_H genes and samples from tissues of healthy persons. Thus, the presence of AMB-1 transcripts comprising Exon 3 (SEQ ID No: 16) correlates to the presence of B-cells with un-mutated IgV_H genes.

Moreover, as described in the as-filed application, the detection of the primary transcript is also restricted to the subtype of B-cells with un-mutated IgV_H genes, thus confirming that spliced AMB-1 transcripts originating from the common un-spliced primary is not detected in mutated IgV_H genes and samples from tissues of healthy persons. By contrast, the absence of any AMB-1 transcript is reflected by the absence of the common AMB-1 primary transcripts.

The absence of any AMB-1 transcript reflected by the absence of the common AMB-1 primary transcripts in tissues of healthy persons is further confirmed in Figure 8, [0245] and [0247] of the present application. Figure 8 shows the overview of the filter used for RNA dot blot probing of a fragment of Exon 3, which comprises 68 different human tissues and 12 human cell lines. The RNA dot blot failed to detect CLLU1 transcript even at very long exposures. Signal was only observed on chromosomal DNA and *E .coli* chromosomal DNA due to contamination of the probe with small traces of E. coli chromosomal DNA.

Therefore, Applicant submits that specification as filed provides that the presence of any AMB-1 transcript, and in particular the nucleic acid sequences recited in the pending claims correlates with B-CLL patients with un-mutated IgV_H genes. The specification also discloses that the un-mutated IgV_H subtype of B-CLL is associated with poor prognosis. Thus, the specification undeniably clearly discloses that the presence of any AMB-1 transcript is associated with poor prognosis and further provides nucleic acid and polypeptide assay detection methods as claimed which could readily be practiced by a person having ordinary skill in the art in order to determine whether an individual .should be diagnosed as having a subtype of B-cell chronic lymphocytic leukemia (B-CLL) associated with a poor prognosis.

It would appear from the final Office Action that the Examiner appreciates the fact that the Northern blot data confirms that the presence of Exon 3 (SEQ IN NO: 16) in the un-mutated subtype of B-cell patients and absence of the same in the mutated subtype of B-cell patients. However, the Examiner states on page 4 1st full paragraph that "*there is no evidence that the entire transcript of SEQ ID NO: 11 is absent from B-cell patients or a subtype of B-cell patients*" (page 4). Also, the Examiner states in the same paragraph that "the blot does not include any control samples or markers it is unclear exactly what the different transcripts that are identified by the Exon 3 probe."

Still further the Examiner states in the following paragraph that "the sequences establish SEQ ID 12-18 when aligned with SEQ ID NO:11 do not produce a consensus sequence, therefore detection of SEQ ID NO:11 would not necessarily detect a region of SEQ ID Nos. 12-18".

Applicants respectfully submit that the statements excerpted from the final Office Action would suggest that the relevance of the data [as explained above and in Applicants' previous response] supporting the efficacy of the claimed detection methods may have been misconstrued. Essentially, and contrary to the apparent basis of the enablement rejection the fact that a primary transcript, identified by a probe comprising a fragment of Exon-3, is present in the unmutated, but absent in the mutated does not refute the efficacy of the claimed detection methods. Rather, this data merely suggests that the resultant spliced transcripts [which would otherwise originate from this Exon] are also absent due to the fact that they originate and therefore will not be expressed unless the parent primary transcript is present.

In particular, the Examiner's statement that *"there is no evidence that the entire transcript of SEQ ID NO: 11 is absent from B-cell patients or a subtype of B-cell patients" (page 4)* would appear to suggest that the structural relationship of SEQ ID NO. 11 to the other sequences recited in the pending claims still may not be clear. [At the outset Applicants note that SEQ ID NO: 11 sequence comprises Exon 3 (SEQ IN NO: 16). Thus, if Exon 3 is absent from the un-mutated subtype of B-cell patients then SEQ ID NO: 11 as such is absent.]

Moreover, the structural relationship between the other detected expressed sequences in the pending claims to SEQ ID NO:11 and based thereon why the data already of record and newly presented establishes the efficacy of the claimed detection methods is further clarified as follows:

Basically, SEQ ID NO: 11 (9.5 kb) is the primary transcript, which comprises SEQ ID NO: 13 (Exon 1), SEQ ID NO: 14 (Exon+3'GT), SEQ ID NO: 15 (Exon 2), SEQ ID NO: 16 (Exon 3), SEQ ID NO: 17 (CDS) which are recited in the pending claims including claim 43. By contrast, the sequences SEQ ID NO: 12 and SEQ ID NO: 18 [previously recited in the claims] prior to the present amendment] are situated down-stream EST Exon, and therefore are not contained in SEQ ID NO: 11. Accordingly, SEQ ID NO: 12 and SEQ ID NO: 18 have been deleted from the amended claim set.

Thus, SEQ ID No: 11 is the parent AMB-1 (CLLU1) transcripts from which all the spliced transcripts of AMB-1 [sequences recited in the claim] originate. Accordingly, the absence of the primary transcript would correlate to the absence of the spliced transcripts.

Therefore, based on the reasons set forth above and in Applicants' prior reply the application contains sufficient evidence and teachings to establish the efficacy and reliability of the claimed methods for detecting a subtype of B-CLL which are based on the detection of specific transcripts. However, in order to further address and refute the enablement rejection a second inventor Declaration is provided as well as supplementary data attached thereto. This data includes the results of a Northern Blot PCR experiments which investigated the relative expression levels of the main CLLU1 transcripts by quantitative PCR (QRT-PCR). This additional data is contained in the two Appendices attached to the newly provided 132 Declaration submitted herewith which is executed by 2 of the inventors.

This new data is exemplary of data substantiating the efficacy and reliability of the claimed detection methods and is submitted in an effort to provide further evidence supporting the efficacy of the claimed methods. However, the declarants, both experts in the relevant art of nucleic acid detection methods, also opine therein that they remain of the opinion that the data and other information already disclosed in the application would sufficiently enable and moreover establish the efficacy of the claimed detection methods.

Turning now to this Declaration and new data, the Northern data (Exhibit A) is exemplary of existing data generated by Applicants relating to the claimed detection methods and was generated prior to the filing date of the PCT application to which this application claims priority and which contains an identical disclosure to said PCT application. As noted in the Declaration, this

Northern corresponds to two phosphor imager images from the same Northern blot which were probed with two different probes, an Exon-3 and a Exon-1 probe. [In order to facilitate comparison of these 2 hybridizations, the blot with probed with Exon-3 is inverted (mirrored).

The Northern was generated using aliquots of RNAs isolated from white blood cells of B-CLL patients were loaded on an agarose gel, which were subjected to electrophoresis. The agarose gel was blotted to a filter: Lane 1-5 (referring to the numbers on the images on the left, Exon-1 probing), 5 RNA samples from 5 patients with CLL and un-mutated Ig genes (poor prognosis); lane 6-8, RNA from 3 patients with CLL and mutated Ig (good prognosis).

The filter (i.e. the Northern blot) was first hybridized with a probe corresponding to Exon-3 (SEQ ID NO: 16). The probe was fully included in Exon-3. After exposure to phosphor imaging the filter was stripped from the Exon-3 probe by boiling and subsequently hybridized with a probe corresponding to Exon-1 (SEQ ID NO: 16), (this probe includes almost all of Exon-1 and nothing else). As can be seen from the data provided, the two probes generate identical hybridization patterns. The identity of the bands is indicated therein: at the top is a band corresponding to the primary transcript, which starts with Exon-1 and ends at the end of Exon-3 without splicing. The two bands correspond to the two versions of the most abundant spliced transcript (Exon-1- Exon-3); the short version utilizes an alternative poly-adenylation site in Exon-3, upstream from the one used in the long form, resulting in a shorter transcript. Thus, this data shows the structural relationship of the detected sequences to SEQ ID NO:11.

In addition, the Declaration further refers to and provides the results of experiments that detected quantitatively the relative expression levels of the main CLLU1 transcripts. This was effected by quantitative PCR (QRT-PCR), using primers that amplified across exon splice sites (for transcripts cDNA1/1a and cDNA1/4) or internal in the putative coding region (CDS). The position of the primers is shown in Fig. 1 of Appendix B of the Declaration. As reported in the Declaration the provided comparisons were made by running QRT-PCR on many patients with the three primer pairs and then comparing the relative expression levels of different transcripts (Figs 2 & 3) (Appendix B).

As evidenced by the supplementary data provided (again exemplary of data substantiating the reliability and efficacy of the claimed detection methods) for all of the transcripts wherein the

expression levels were quantified the inventors observed a linear correlation between the expression levels of the different transcripts, i.e. the relative patient-dependent expression level of CLLU1 was independent of which transcript we quantified. These linear expression levels are not coincidental and can only be explained by the presence of a primary transcript that is spliced (or un-spliced) to the detected mRNAs, which are present in different relative amounts depending on the efficiency of the individual splicing reactions.

As explained by the Declarants the efficiency of such splicing reactions is determined by the sequences flanking the splice sites and is therefore patient independent. Thus, the transcript corresponding to cDNA1 (exon1-exon3) is the most efficiently spliced transcript and is therefore present in largest amount in all the patients and accordingly is detectable with lower ΔCt numbers than the less efficiently produced (spliced) transcript corresponding to cDNA2/4 in Fig. 2. Since the ΔCt values were normalized to the levels in B-cells in Fig. 3, the two transcripts (cDNA1 and CDS) appear with equal values because the splicing efficiency (i.e. splicing of exon1-3 and lack of splicing for CDS) of the two transcripts as expected is conserved between CLL- and B-cells. However, the actual level of cDNA1 is in all samples in fact much higher than the actual level of mRNA containing the CDS (as seen in Fig. 2 for cDNA1 and cDNA2/4).

Applicants respectfully submit that this data should further substantiate the structural relationship of the detected transcripts to SEQ ID NO:11 and why detecting the expression thereof as claimed herein may be used to detect whether a particular individual has a particular virulent subtype of B-CLL. No data or scientific reasoning raised by the Examiner is believed to refute the efficacy of the claimed invention. Based on this additional data, and further based upon the data and arguments already of record, the undersigned respectfully submit that the rejection under 35 U.S.C. 112, first paragraph as lacking enablement should be withdrawn.

In Applicant's view, the evidence supporting the effcicay of the claimed detection methods is overwhelming. Moreover, even absent the additional data, for the reasons already of record Applicants submit that the efficacy of the claimed detection methods which essentially rely on the principle of a particular primary transcript being spliced to mRNA to produce different spliced variants (the expression of which is detected) is apparent from the as-filed application and the data therein. As previously argued it would be readily understood by a skilled artisan that the absence of a particular primary transcript (which correlates to a specific subtype of B-CLL) would in turn mean

that no spliced transcripts (derived therefrom) will be generated and therefore will not be detected if the primary transcript is absent..

Based on the foregoing, Applicants respectfully submit that this application should be passed to issue as the only rejection is unsupportable. Essentially, the specification alone or coupled wit the supplementary data included with the Declaration submitted herewith would clearly establish to a person skilled in the art that detecting the presence of "any AMB-1 transcript" correlates with the un-mutated IgV_H genes subtype of B-CLL.

Also, this case should be expeditiously allowed given the utmost importance of an improved method for detecting a virulent subtype of B-CLL and its prognosis, a condition which afflicts and kills many individuals annually. This invention provides such an improvement because it reliably identifies individuals with this disease and their prognosis relative to other individuals (who do not express this subtype of the disease). Such detection will facilitate the design and earlier implementation of appropriate and improved therapeutic regimens based on this diagnosis.

No additional fees are believed to be due for this amendment. However, the Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. § 1.16 and § 1.17 associated with this communication or credit any overpayment to the deposit account of Hunton & Williams, **Deposit Account Number 50-0206**.

Respectfully submitted,

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Dated: January 14, 2009 By: _____

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